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## ACCEPTED MANUSCRIPT

# Purification, characterization and expression of transferrin from rainbow trout seminal plasma

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#### **Abstract**

Transferrin (TF) is recognized as a multifunctional protein and has been implicated in antioxidative, antimicrobial protection, growth, differentiation and cytoprotection effects. An efficient, original three-step isolation procedure for TF consisting in hydrophobic interaction chromatography, gel filtration and preparative electrophoresis was developed. Rainbow trout TF was found to be N-glycosylated (not O-glycosylated) and phosphorylated at all serine, threonine, and tyrosine resides. The protein consists of several proteoforms with an average molecular weight of 76.9 kDa and isoelectric point ranging from 5.2 to 5.7. Rainbow trout TF has two functional iron-binding sites and appears to be quite distinct from carp TF regarding glycosylation and iron-binding properties. The highest gene expression of TF was detected in liver and testis, the lowest was detected in head kidney, spleen and efferent ducts. For the first time TF was identified in the semen of several salmonid species. TF was localized within testis, mainly in spermatozoa, Sertoli, Leydig cells, as well as in both columnar secretory and basal cells within the efferent duct. This work contributes to the existing knowledge information indicating significant variations in TF structure within teleost fish. The results obtained in this study provide valuable data on the TF from trout seminal plasma and the physiological role of this protein in the reproductive tract of salmonids. The results are important for our understanding of the role of TF in the antioxidant protection and resistance to pathogenic

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